

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 9/51, 9/14, 49/04	A1	(11) International Publication Number: WO 98/07414 (43) International Publication Date: 26 February 1998 (26.02.98)
(21) International Application Number: PCT/US97/04695 (22) International Filing Date: 28 March 1997 (28.03.97) (30) Priority Data: 08/701,483 22 August 1996 (22.08.96) US (71) Applicant: RESEARCH TRIANGLE PHARMACEUTICALS LTD. [US/US]; 4364 South Alston Avenue, Durham, NC 27713-2280 (US). (72) Inventors: PARIKH, Indu; 2558 Booker Creek Road, Chapel Hill, NC 27514 (US). SELVARAJ, Ulagaraj; 5323-C Penrith Drive, Durham, NC 27713 (US). (74) Agent: CRAWFORD, Arthur, R.; Nixon & Vanderhye P.C., 8th floor, 1100 North Glebe Road, Arlington, VA 22201-4714 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: COMPOSITIONS COMPRISING MICROPARTICLES OF WATER-INSOLUBLE SUBSTANCES AND METHOD FOR PREPARING SAME (57) Abstract Submicron size particles of pharmaceutical or other water-insoluble or poorly water-insoluble substances are prepared using a combination of one or more surface modifiers/surfactants such as polaxomers, poloxamines, polyoxyethylene sorbitan fatty acid esters and the like together with natural or synthetic phospholipids. Particles so produced have a volume weighted mean particle size at least one-half smaller than obtainable using a phospholipid alone. Compositions so prepared are resistant to particle size growth on storage.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

**COMPOSITIONS COMPRISING MICROPARTICLES OF WATER-INSOLUBLE SUBSTANCES AND
METHOD FOR PREPARING SAME**

This invention relates to compositions and procedures that yield
5 sub-micron and micron stable particles of water-insoluble or poorly
soluble drugs or other industrially useful insoluble compounds. The
compositions of this invention include combinations of natural or
synthetic phospholipids, and one or more non-ionic, anionic or
cationic surfactants coated or adhered onto the surfaces of the water
10 insoluble-compound particles. The combination of phospholipids and
surfactants allows the formation and stabilization of the sub-micron
and micron size compound particles via hydrophilic, lipophilic and
electrostatic interactions and therefore prevent these particles from
aggregation or flocculation.

15

BACKGROUND OF THE INVENTION

There is a critical need in the pharmaceutical and other
biological based industries to formulate water-insoluble or poorly
20 soluble substances into formulations for oral, injectable, inhalation
and ophthalmic routes of delivery. Water insoluble compounds are
those having poor solubility in water, that is < 5 mg/ml at
physiological pH (6.5-7.4). Preferably their water solubility is $<$
1 mg/ml, more preferably < 0.1 mg/ml. It is desirable that the drug is
25 stable in water as a dispersion; otherwise a lyophilized or spray-dried
solid form may be desirable.

As used herein, "micro" refers to a particle having diameter of from nanometers to micrometers. Microparticles, as used herein, refer to solid particles of irregular, non-spherical or spherical shapes. Formulations containing these microparticles provide some specific advantages over the unformulated non-micronized drug particles, which include improved oral bioavailability of drugs that are poorly absorbed from GI tract, development of injectable formulations that are currently available only in oral dosage form, less toxic injectable formulations that are currently prepared with organic solvents, sustained release of intramuscular injectable drugs that are currently administered through daily injection or constant infusion, and preparation of inhaled, ophthalmic formulation of drugs that otherwise could not be formulated for nasal or ocular use.

Current technology for delivering insoluble drugs as described in US Patents 5,091,188; 5,091,187 and 4,725,442 focuses on (a) either coating small drug particles with natural or synthetic phospholipids or (b) dissolving the drug in a suitable lipophilic carrier and forming an emulsion stabilized with natural or semisynthetic phospholipids. One of the disadvantages of these formulations is that certain drug particles in suspension tend to grow over time because of the dissolution and reprecipitation phenomenon known as the "Oswald ripening".

DESCRIPTION OF THE INVENTION

The present invention focuses on preparing submicron size particles using a combination of surface modifier(s) with a phospholipid, and how the growth of particle size, and hence storage stability, is

controlled by adding a combination of surface modifier(s) with a phospholipid to the formulation.

The use of a surface modifier or combination of surface
5 modifiers in addition to a phospholipid is characterized by its ability to result in volume weighted mean particle size values that are (i) at least 50% and preferably about 50-90% smaller than what can be achieved using phospholipid alone without the use of a surfactant with the same energy input, and (ii) provide compositions resistant to
10 particle size growth on storage. While resistance to particle size growth on storage was an objective of this invention we were surprised to observe a significant reduction in particle size with the addition of the surfactant. In order to achieve the advantages of the present invention it is necessary that the phospholipid and the
15 surfactant both be present at the time of particle size reduction or precipitation.

Although we do not wish to be bound by any particular theory, it appears that these surface modifiers generally, that is phospholipids
20 and one or more surfactants, adsorb to the surfaces of drug particles. and (a) convert lipophilic to hydrophilic surfaces with increased steric hindrance/stability, and (b) possibly modify zeta potential of surfaces with more charge repulsion stabilization. The concentrations of surface modifiers used in the process described here are normally
25 above their critical micelle concentrations (CMC) and hence facilitate the formation of sub-micron particles by stabilizing the particles.

Phospholipid and surface modifier(s) are adsorbed on to the surfaces of drug particles in sufficient quantity to retard drug particle growth, reduce drug average particle size from 5 to 100 μm to sub-micron and micron size particles by one or combination of methods
5 known in the art, such as sonication, homogenization, milling, microfluidization, precipitation or recrystallization or precipitation from supercritical fluid, and maintain sub-micron and micron size particles on subsequent storage as suspension or solid dosage form.

10 The concentration of phospholipid or surface modifier in the suspension or solid dosage form can be present in the range of 0.1 to 50%, preferably 0.2 to 20%, and more preferably 0.5 to 10%.

The formulations prepared by this invention may be lyophilized
15 into powders, which can be resuspended or filled into capsules or converted into granules or tablets with the addition of binders and other excipients known in the art of tablet making.

By industrially useful insoluble or poorly soluble compounds
20 we include biologically useful compounds, imaging agents, pharmaceutically useful compounds and in particular drugs for human and veterinary medicine. Water insoluble compounds are those having a poor solubility in water, that is less than 5 mg/ml at a physiological pH of 6.5 to 7.4, although the water solubility may be
25 less than 1 mg/ml and even less than 0.1 mg/ml.

Examples of some preferred water-insoluble drugs include immunosuppressive and immunoactive agents, antiviral and

antifungal agents, antineoplastic agents, analgesic and anti-inflammatory agents, antibiotics, anti-epileptics, anesthetics, hypnotics, sedatives, antipsychotic agents, neuroleptic agents, antidepressants, anxiolytics, anticonvulsant agents, antagonists, 5 neuron blocking agents, anticholinergic and cholinomimetic agents, antimuscarinic and muscarinic agents, antiadrenergic and antarrhythmics, antihypertensive agents, antineoplastic agents, hormones, and nutrients. A detailed description of these and other suitable drugs may be found in *Remington's Pharmaceutical Sciences*, 10 18th edition, 1990, Mack Publishing Co. Philadelphia, PA.

The phospholipid may be any natural or synthetic phospholipid. for example phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol. 15 phosphatidic acid, lysophospholipids, egg or soybean phospholipid or a combination thereof. The phospholipid may be salted or desalted, hydrogenated or partially hydrogenated or natural semisynthetic or synthetic.

20 Examples of some suitable second surface modifiers include:
(a) natural surfactants such as casein, gelatin, tragacanth, waxes, enteric resins, paraffin, acacia, gelatin, cholesterol esters and triglycerides, (b) nonionic surfactants such as polyoxyethylene fatty alcohol ethers, sorbitan fatty acid esters, polyoxyethylene fatty acid 25 esters, sorbitan esters, glycerol monostearate, polyethylene glycols, cetyl alcohol, cetostearyl alcohol, stearyl alcohol, poloxamers, polaxamines, methylcellulose, hydroxycellulose, hydroxy propylcellulose, hydroxy propylmethylcellulose, noncrystalline

cellulose, polyvinyl alcohol, polyvinylpyrrolidone, and synthetic phospholipids, (c) anionic surfactants such as potassium laurate, triethanolamine stearate, sodium lauryl sulfate, alkyl polyoxyethylene sulfates, sodium alginate, dioctyl sodium sulfosuccinate, negatively charged phospholipids (phosphatidyl glycerol, phosphatidyl inositol, phosphatidylserine, phosphatidic acid and their salts), and negatively charged glyceryl esters, sodium carboxymethylcellulose, and calcium carboxymethylcellulose, (d) cationic surfactants such as quaternary ammonium compounds, benzalkonium chloride, cetyltrimethylammonium bromide, chitosans and lauryldimethylbenzylammonium chloride, (e) colloidal clays such as bentonite and veegum. A detailed description of these surfactants may be found in Remington's Pharmaceutical Sciences, and Theory and Practice of Industrial Pharmacy, Lachman et al, 1986.

More specifically, examples of suitable second surface modifiers include one or combination of the following: polaxomers, such as Pluronic™ F68, F108 and F127, which are block copolymers of ethylene oxide and propylene oxide available from BASF, and poloxamines, such as Tetronic™ 908 (T908), which is a tetrafunctional block copolymer derived from sequential addition of ethylene oxide and propylene oxide to ethylene-diamine available from BASF, Triton™ X-200, which is an alkyl aryl polyether sulfonate, available from Rohm and Haas. Tween 20, 40, 60 and 80, which are polyoxyethylene sorbitan fatty acid esters, available from ICI Speciality Chemicals, Carbowax™ 3550 and 934, which are polyethylene glycols available from Union Carbide, hydroxy propylmethylcellulose, dimyristoyl phosphatidylglycerol sodium salt,

sodium dodecylsulfate, sodium deoxycholate, and cetyltrimethylammonium bromide.

It is thought that some of the functions of the second surface
5 modifier(s) as it relates to this invention are suppressing the process
of Oswald Ripening and therefore maintaining the particle size,
increasing the storage stability, minimizing sedimentation, and
decreasing the particle growth during lyophilization and
reconstitution; adhere or coat firmly onto the surfaces of
10 water-insoluble drug particles and therefore modify the interfaces
between the particles and the liquid in the resulting formulations;
increase the interface compatibility between water-insoluble drug
particles and the liquid; and possibly to orient preferentially
themselves with the hydrophilic portion sticking into the aqueous
15 solution and the lipophilic portion strongly adsorbed at the
water-insoluble drug particle surfaces

Considerable variations as to the identities and types of
phospholipid and especially the surface active agent or agents should
20 be expected depending upon the drug or active agent selected as the
surface properties of these small particles are different. The most
advantageous surface active agent for the insoluble drug will be
apparent following empirical tests to identify the surfactant or
surfactant system/combination resulting in the requisite particle size
25 and particle size stability on storage over time.

Various procedures can be used to produce these stable
sub-micron and micron size particles including mixing the insoluble

substance with phospholipid and precipitating from a dissolved mixture of the substance, phospholipid and surfactant using other surfactants followed by sonication, milling, homogenization, microfluidization, and antisolvent and solvent precipitation. Mannitol
5 and other agents may be added to adjust the final formulation to isotonicity as well as a stabilizing aid during drying.

Unless otherwise specified, all parts and percentages reported herein are weight per unit volume (w/v), in which the volume in the
10 denominator represents the total volume of the system. Diameters of dimensions are given in millimeters ($\text{mm} = 10^{-3}$ meters), micrometers ($\mu\text{m} = 10^{-6}$ meters), nanometers ($\text{nm} = 10^{-9}$ meters) or Angstrom units ($= 0.1 \text{ nm}$). Volumes are given in liters (L), milliliters ($\text{mL} = 10^{-3} \text{ L}$) and microliters ($\mu\text{L} = 10^{-6} \text{ L}$). Dilutions are by volume. All
15 temperatures are reported in degrees Celsius. The compositions of the invention can comprise, consist essentially of or consist of the materials set forth and the process or method can comprise, consist essentially of or consist of the steps set forth with such materials.

20 The following examples further explain and illustrate the invention:

Example 1

25 Microparticle-cyclosporine, of an immunosuppressive drug, was prepared as follows. The composition and concentration of excipients of the microparticle cyclosporine formulation are listed below:

	Cyclosporine	50 mg/ml
	Egg Phosphatidylcholine	100 mg/ml
	Mannitol	55 mg/ml
	Tween 80	10 mg/ml
5	Distilled Water	qs to 100%
	Total Volume	20 ml

Cyclosporine with an average particle size from 5-100 μm , and mannitol were purchased from Sigma, egg phosphatidylcholine was
10 produced by Pfanstiehl, Tween 80 was purchased from ICI.

The above components were placed in a 30 ml beaker and pre-mixed with a hand-held biohomogenizer (Honeywell DR 4200 model GP) for 1-5 min. During homogenization, dilute NaOH was
15 added to the pre-mix to adjust the pH from 3.1 to 7 ± 0.5 . The pre-mix was placed in a water jacketed vessel (50 ml capacity) through which thermostated water at 4°C was circulated to control the temperature of the formulation. The pre-mix was subjected to high shear energy of a probe sonicator (Fisher, model 550 Sonic
20 Dismembrator) with a 0.5 inch diameter probe. Sonic pulses of 10 seconds at 10-seconds intervals at a power setting of 5 were utilized. During sonication the temperature of the formulation was $18 \pm 2^\circ\text{C}$. The pH during sonication was adjusted to 7 ± 0.5 with dilute NaOH. Total sonication time employed to prepare the microparticle
25 cyclosporine was usually 10.5 hours or less. The microparticle-cyclosporine formulation was placed in 20 ml vials and stored at 4 and 25°C for further stability studies.

Particle size distribution of the suspension was analyzed with a NICOMP model 370 Particle Size Analyzer. This instrument utilizes photon correlation spectroscopy for particle sizing in the submicron region. A small volume of the suspension was diluted with water and placed in the cell of the particle size analyzer. Particle size determination based on volume weighted and number weighted particle size determination of the suspension, represented as a Gaussian distribution by the NICOMP 370 software, yielded the mean particle size values, which are listed below in Table I.

10

Table I: Volume-and Number-weighted Particle Size Stability of Microparticle-Cyclosporine

Storage Time	Storage at 4°C		Storage at 25°C	
	Mean Particle Size (nm)		Mean Particle Size (nm)	
Days	Volume-Weighted	Number-Weighted	Volume-Weighted	Number-Weighted
0	361	63	361	63
7	337	69	423	67
51	358	76	455	66

20

Approximately 20 μ l of the freshly prepared suspension was placed on a clean slide, with a clean cover glass, and examined under an Olympus BH2 microscope with 1000X magnification. An eye-piece equipped with a graticule was used to estimate the particle size. Most of the particles in the suspension were 0.3-0.5 μ m.

25

Furthermore, microscopic examination of the suspension confirmed non-agglomerated or flocculated micron and sub-micron size drug particles exhibiting Brownian motion.

5

Example 2

For purpose of comparison (not according to the invention) using only a phospholipid, microparticle-cyclosporine with lecithin alone (without the second surface modifier, Tween 80) was also prepared using the same procedure as Example 1. The suspension was stored in 20 ml glass vials for storage stability studies. The volume and number weighted mean particle size values of the suspension stored at 4 and 25°C are listed below. The results in Table II illustrate that the presence of lecithin alone (without the presence of Tween 80) does not provide the particle size reduction and enhancement in storage stability as described in Example 1.

Table II: Volume-weighted Particle Size Stability of Microparticle-Cyclosporine

20

Storage Time	Storage at 4°C		Storage at 25°C	
	Mean Particle Size (nm)		Mean Particle Size (nm)	
Days	Volume-Weighted	Number-Weighted	Volume-Weighted	Number-Weighted
0	704	91	704	91
1	1472	503	2230	755
6	1740	416	2290	874

25

Example 3

For purpose of comparison (not according to the invention) using only a surface modifier, microparticle-cyclosporine with Tween 80 alone (without a phospholipid, egg phosphatidylcholine) was also prepared using the same procedure as Example 1. The suspension was stored in 20 ml glass vials. The results in Table III illustrate that the presence of Tween 80 alone (without the presence of phospholipid) does not provide particle size reduction as in Example 1.

Table III: Volume- and Number-weighted Particle Size Stability of Microparticle-Cyclosporine

Mean Particle Size (nm)		
Day	Volume-Weighted	Number-Weighted
0	521	67

Example 4

The following microparticle-Docosanol formulations were prepared by the process of the invention with Tween 80, Tween 20, egg phosphatidylcholine, and/or Phospholipon 90H as surface modifiers. Docosanol is available from Sigma. The formulations were prepared according to the procedures of Example 1. The compositions and concentration of excipients of the microparticle formulations are listed below:

Microparticle-Docosanol (Example 4.1, comparative)

	Docosanol	20 mg/ml
	Egg Phosphatidylcholine	50 mg/ml
5	Mannitol	55 mg/ml
	Distilled Water	qs to 100%
	Total Volume	20 ml

Microparticle-Docosanol (Example 4.2)

10

	Docosanol	20 mg/ml
	Egg Phosphatidylcholine	50 mg/ml
	Mannitol	55 mg/ml
	Tween 80	10 mg/ml
15	Distilled Water	qs to 100%
	Total Volume	20 ml

Microparticle-Docosanol (Example 4.3)

20

	Docosanol	20 mg/ml
	Egg Phosphatidylcholine	50 mg/ml
	Mannitol	55 mg/ml
	Tween 20	10 mg/ml
25	Distilled Water	qs to 100%
	Total Volume	20 ml

Microparticle-Docosanol (Example 4.4)

	Docosanol	20 mg/ml
	Phospholipon 90H	30 mg/ml
5	Mannitol	55 mg/ml
	Tween 80	10 mg/ml
	Distilled Water	qs to 100%
	Total Volume	20 ml

10 Microparticle-Docosanol (Example 4.5, Comparative)

	Docosanol	20 mg/ml
	Mannitol	55 mg/ml
	Tween 80	10 mg/ml
15	Distilled Water	qs to 100%
	Total Volume	20 ml

The mean volume-and number-weighted particle size values of the suspension were 286 nm, and 98 nm, respectively.

20

The volume weighted mean particle size values of the above suspension stored at 4°C are listed below in Table IV.

Table IV: Volume-weighted and Number Weighted Particle Size Stability of Microparticle-Docosanol Stored at 4° C.

Storage Time	(Example 4.1)		(Example 4.2)	
	Mean Particle Size (nm)		Mean Particle Size (nm)	
Days	Volume-Weighted	Number-Weighted	Volume-Weighted	Number-Weighted
0	688	--	112	55
30	ND	ND	156	81

Storage Time	(Example 4.3)		(Example 4.4)	
	Mean Particle Size (nm)		Mean Particle Size (nm)	
Days	Volume-Weighted	Number-Weighted	Volume-Weighted	Number-Weighted
0	129	61	90	35
30	184	99	127	39

ND = Not Determined

The above data illustrate the much smaller particles produced by the present invention with the presence of a surfactant in addition to the phospholipid and that these particles retain their particle size over time without significant increase in size.

Example 5

The following seven microparticle-RTP-4055 (an antiviral drug) formulations were prepared with combinations of Tween 80, Tetronic 908, Pluronic F-68, egg phosphatidylcholine, and/or phospholipon 90H as surface modifiers. The details of the sonication method are similar to those discussed in Example 1. The compositions and concentration of excipients of the microparticle formulations are listed below:

10

Microparticle-RTP-4055 (Example 5.1, Comparative)

	RTP-4055	50 mg/ml
	Egg Phosphatidylcholine	50 mg/ml
15	Distilled Water	qs to 100%
	Total Volume	25 ml

The mean volume weighted particle size of the suspension was 3195 nm.

20

Microparticle-RTP-4055 (Example 5.2)

	RTP-4055	50 mg/ml
	Egg Phosphatidylcholine	50 mg/ml
25	Mannitol	55 mg/ml
	Pluronic F-68	5 mg/ml
	Distilled Water	qs to 100%
	Total Volume	25 ml

The mean volume- and number-weighted particle size values of the suspension were 672 nm and 76 nm respectively.

5 Microparticle-RTP-4055 (Example 5.3)

	RTP-4055	50 mg/ml
	Egg Phosphatidylcholine	50 mg/ml
	Mannitol	55 mg/ml
10	Tetronic 908	5 mg/ml
	Distilled Water	qs to 100%
	Total Volume	25 ml

The mean volume- and number- weighted particle size values of the
15 suspension were 436 nm and 59 nm respectively.

Microparticle-RTP-4055 (Example 5.4, Comparative)

	RTP-4055	50 mg/ml
20	Phospholipon 90H	30 mg/ml
	Distilled Water	qs to 100%
	Total Volume	25 ml

The mean volume- number- weighted particle size values of the
25 suspension were 1117 nm. and 108 nm respectively.

Microparticle-RTP-4055 (Example 5.5)

	RTP-4055	50 mg/ml
	Phospholipon 90H	30 mg/ml
5	Mannitol	55 mg/ml
	Dimyristoylphosphatidyl choline (DMPG)	3 mg/ml
	Tween 80	10 mg/ml
	Distilled Water	qs to 100%
10	Total Volume	25 ml

The mean volume weighted particle size of the suspension was 236 nm. The particle size of the suspension stored at 4°C for 1 week and 1 month are 328 and 397 nm, respectively, which indicates the
15 stability of the suspension.

Microparticle-RTP-4055 (Example 5.6)

	RTP-4055	50 mg/ml
20	Phospholipon 90H	30 mg/ml
	Mannitol	55 mg/ml
	Tween 80	10 mg/ml
	Distilled Water	qs to 100%
	Total Volume	25 ml

25

The mean volume- and number- weighted particle size values of the suspension were 382 nm and 59 nm respectively. Within the

error limits, there was no variation in the mean particle size after one week of storage at 4°C.

Microparticle-RTP-4055 (Example 5.7, Comparative)

5	RTP-4055	50 mg/ml
	Mannitol	55 mg/ml
	Tween 80	10 mg/ml
	Distilled Water	qs to 100%
10	Total Volume	25 ml

The volume- and number-weighted mean particle size values of the suspension were 545 nm, and 75 nm, respectively within the error limits, there was no variation in the mean particle size after one week
15 of storage at 4°C.

Example 6

20 The following six microparticle-Piroxicam formulations were prepared with combination of Tween 80, Tetronic 908, Pluronic F-68, and/or egg phosphatidylcholine as surface modifiers. Piroxicam was received from Cipla. The details of the sonication method are similar to those discussed in example 1. The compositions and concentration
25 of excipients of the microparticle formulations are listed below:

Microparticle-Piroxicam (Example 6.1)

	Piroxicam	67 mg/ml
	Egg Phosphatidylcholine	67 mg/ml
5	Mannitol	67 mg/ml
	Tween 80	5 mg/ml
	Tetronic 908	5 mg/ml
	Distilled Water	qs to 100% (w/v)
	Total Volume	15 ml

10

The mean volume- and number- weighted particle size values of the suspension were 674 nm and 72 nm respectively.

Microparticle-Piroxicam (Example 6.2)

15

	Piroxicam	67 mg/ml
	Egg Phosphatidylcholine	67 mg/ml
	Mannitol	67 mg/ml
	Tetronic 908	5 mg/ml
20	Distilled Water	qs to 100% (w/v)
	Total Volume	15 ml

The mean volume- and number- weighted particle size values of the suspension were 455 nm and 58 nm respectively.

25

Microparticle-Piroxicam (Example 6.3)

	Piroxicam	67 mg/ml
	Egg Phosphatidylcholine	67 mg/ml
5	Mannitol	67 mg/ml
	Pluronic F-68	5 mg/ml
	Distilled Water	qs to 100% (w/v)
	Total Volume	15 ml

10 The mean volume- and number- weighted particle size values of the suspension were 564 nm and 68 nm respectively.

Microparticle-Piroxicam (Example 6.4)

15	Piroxicam	67 mg/ml
	Egg Phosphatidylcholine	67 mg/ml
	Mannitol	67 mg/ml
	Tween 80	5 mg/ml
20	Cetyltrimethylammonium bromide	10 mg/ml
	Distilled Water	qs to 100% (w/v)
	Total Volume	15 ml

25 The mean volume- and number- weighted particle size values of the suspension were 479 nm and 80 nm respectively.

Microparticle-Piroxicam (Example 6.5)

	Piroxicam	67 mg/ml
	Egg Phosphatidylcholine	67 mg/ml
5	Mannitol	67 mg/ml
	Cetyltrimethylammonium bromide	10 mg/ml
	Distilled Water	qs to 100% (w/v)
10	Total Volume	15 ml

The mean volume- and number- weighted particle size values of the suspension were 670 nm and 128 nm respectively.

15 Microparticle-Piroxicam (Example 6.6, Comparative)

	Piroxicam	67 mg/ml
	Mannitol	67 mg/ml
	Tween 80	5 mg/ml
20	Tetronic 908	5 mg/ml
	Distilled Water	qs to 100%
	Total Volume	25 ml

The volume- and number- weighted particle size values of the
25 suspension were 1184 nm and 385 nm, respectively.

WHAT IS CLAIMED IS:

3 1. A composition of microparticles of a water-insoluble
4 substance comprising particles of an industrially useful water-
5 insoluble or poorly soluble compound, a phospholipid and at least one
6 non-ionic, anionic or cationic surfactant, in which the surfactant or
7 surfactants provide volume-weighted mean particle size values of the
8 water-insoluble compound at least 50% smaller than particles
9 produced without the presence of the surfactant using the same energy
10 input.

1 2. A pharmaceutical composition of microparticles of a water-
2 insoluble substance comprising particles of an industrially useful
3 water-insoluble or poorly soluble compound, a phospholipid and at
4 least one non-ionic, anionic or cationic surfactant, in which the
5 surfactant or surfactants provide volume-weighted mean particle size
6 values of the water-insoluble compound at least 50% smaller than
7 particles produced without the presence of the surfactant using the
8 same energy input.

1 3. The pharmaceutical composition of claim 2 for oral,
2 inhalation, ocular, nasal or injectable administration.

1 4. The pharmaceutical composition of claim 3 in injectable
2 form for intravenous, intra-arterial, intra-muscular, intradermal,
3 subcutaneous, intra-articular, cerebrospinal, epidural, intracostal,
4 intraperitoneal, intratumor, intrabladder, intra-lesion or
5 subconjunctival administration.

1 5. A dried suspension of the composition of claim 4 which can
2 be resuspended in aqueous or non-aqueous media.

1 6. A suspension, spray-dried powder, lyophilized powder
2 granules or tablets of the composition of claim 2.

1 7. A composition of claim 1 in which the water-insoluble
2 compound is a biologically useful compound or an imaging agent.

1 8. The composition of claim 1 or claim 2 wherein the
2 surfactant is a polyoxyethylene sorbitan fatty acid ester, a block
3 copolymer of ethylene oxide and propylene oxide, a tetrafunctional
4 block copolymer derived from sequential addition of ethylene oxide
5 and propylene oxide to ethylenediamine, an alkyl aryl polyether
6 sulfonate, polyethylene glycol, hydroxy propylmethylcellulose,
7 sodium dodecylsulfate, sodium deoxycholate,
8 cetyltrimethylammonium bromide or combinations thereof.

1 9. The process of claim 1 or 2 wherein the phospholipid is of
2 egg or plant origin or semisynthetic or synthetic in partly or fully
3 hydrogenated form or in a desalted or salt form such as
4 phosphatidylcholine, phospholipon 90H or dimyristoyl
5 phosphatidylglycerol sodium salt, phosphatidylethanolamine,
6 phosphatidylserine, phosphatidic acid, lysophospholipids or
7 combinations thereof.

1 10. A process for preparing sub-micron and micron sized,
2 stable particles of water-insoluble or a poorly soluble industrially
3 useful compound using natural or synthetic phospholipids, said
4 process comprising reducing the particle size by sonication,
5 homogenization, milling, microfluidization and precipitation, or
6 recrystallization and precipitation of the compound using antisolvent
7 and solvent precipitation including from supercritical fluids in the
8 presence of a phospholipid and at least one non-ionic, anionic or
9 cationic surfactant.

1 11. A process of preparing microparticles of a water-insoluble
2 or poorly soluble compound comprising the steps of:
3 (1) mixing particles of a water-insoluble or poorly soluble
4 industrially useful compound with a phospholipid and at least one
5 non-ionic, anionic or cationic surfactant, and thereafter
6 (2) applying energy to the mixture sufficient to produce
7 volume-weighted mean particle size values of the compound at least
8 50% smaller than particles produced without the presence of the
9 surfactant using the same energy input.

1 12. The process of claim 10 or 11 wherein the phospholipid is
2 of egg or plant origin or semisynthetic or synthetic in partly or fully
3 hydrogenated form or in a desalted or salt form such as
4 phosphatidylcholine, phospholipon 90H or dimyristoyl
5 phosphatidylglycerol sodium, salt, phosphatidylethanolamine,
6 phosphatidylserine, phosphatidic acid, lysophospholipids, or
7 combinations thereof.

1 13. The process of claim 10 or 11 wherein the surfactant is a
2 polyoxyethylene sorbitan fatty acid ester, a block copolymer of
3 ethylene oxide and propylene oxide, a tetrafunctional block
4 copolymer derived from sequential addition of ethylene oxide and
5 propylene oxide to ethylenediamine, an alkyl aryl polyether sulfonate,
6 polyethylene glycol, hydroxy propylmethylcellulose, sodium
7 dodecylsulfate, sodium deoxycholate, cetyltrimethylammonium
8 bromide or combinations thereof.

1 14. The process of claim 10 or 11 wherein the surfactant is
2 present above the critical micelle concentration.

1 15. The process of claim 10 or 11 in which the compound is a
2 biologically useful compound or an imaging agent.

1 16. A composition comprising microparticles prepared by the
2 process of claim 10.

1 17. A composition comprising microparticles produced by the
2 process of claim 11.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/04695

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/51 A61K9/14 A61K49/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 601 618 A (STERLING WINTHROP INC) 15 June 1994 see the whole document ---	1-4, 6-13, 15-17
X	EP 0 602 700 A (STERLING WINTHROP INC) 22 June 1994 see the whole document ---	1-4, 6-9
X	US 5 447 710 A (NA GEORGE C ET AL) 5 September 1995 see the whole document ---	1-4, 6-9
X	US 5 326 552 A (NA GEORGE C ET AL) 5 July 1994 see the whole document ---	1-4, 6-9
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

2 December 1997

Date of mailing of the international search report

15/12/1997

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 551 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Fischer, W

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/04695

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E, L	WO 97 14407 A (RES TRIANGLE PHARMACEUTICALS ; UNIV TEXAS (US); HENRIKSEN INGE B (U) 24 April 1997 "L": DOCUMENT SO QUOTED FOR ITS' CASTING DOUBT ON THE VALIDITY OF THE CONVENTION-PRIORITY CLAIMED see the whole document ---	1-4, 6-13, 15-17
A	US 5 091 187 A (HAYNES DUNCAN H) 25 February 1992 ---	
A	US 5 364 633 A (HILL RANDAL M ET AL) 15 November 1994 ---	
A	WO 94 20072 A (PHARMACIA AB ; WESTESEN KIRSTEN (DE); SIEKMANN BRITTA (DE)) 15 September 1994 -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/04695

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0601618 A	15-06-94	US 5336507 A	09-08-94
		AU 662453 B	31-08-95
		AU 5046893 A	23-06-94
		CA 2102267 A	12-06-94
		CZ 9302602 A	15-06-94
		FI 935305 A	12-06-94
		HU 65758 A	28-07-94
		JP 6211646 A	02-08-94
		NO 934204 A	13-06-94
		NZ 250062 A	27-04-95
		SK 139093 A	07-12-94
		US 5470583 A	28-11-95
EP 0602700 A	22-06-94	US 5326552 A	05-07-94
		AU 664115 B	02-11-95
		AU 4867293 A	30-06-94
		CA 2107165 A	18-06-94
		CZ 9302668 A	17-08-94
		FI 935396 A	18-06-94
		HU 67265 A	28-03-95
		JP 6192131 A	12-07-94
		MX 9306012 A	31-01-95
		NO 934425 A	20-06-94
		NZ 248727 A	27-04-95
		SK 142793 A	06-07-94
US 5447710 A	05-09-95	US 5326552 A	05-07-94
		AU 664115 B	02-11-95
		AU 4867293 A	30-06-94
		CA 2107165 A	18-06-94
		CZ 9302668 A	17-08-94
		EP 0602700 A	22-06-94
		FI 935396 A	18-06-94
		HU 67265 A	28-03-95
		JP 6192131 A	12-07-94
		MX 9306012 A	31-01-95
		NO 934425 A	20-06-94
		NZ 248727 A	27-04-95
		SK 142793 A	06-07-94

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/04695

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5326552 A	05-07-94	AU 664115 B	02-11-95
		AU 4867293 A	30-06-94
		CA 2107165 A	18-06-94
		CZ 9302668 A	17-08-94
		EP 0602700 A	22-06-94
		FI 935396 A	18-06-94
		HU 67265 A	28-03-95
		JP 6192131 A	12-07-94
		MX 9306012 A	31-01-95
		NO 934425 A	20-06-94
		NZ 248727 A	27-04-95
		SK 142793 A	06-07-94
		US 5447710 A	05-09-95
WO 9714407 A	24-04-97	AU 7461796 A	07-05-97
US 5091187 A	25-02-92	US 5091188 A	25-02-92
		AU 7852891 A	11-11-91
		CA 2078990 A	27-10-91
		EP 0533690 A	31-03-93
		IN 173056 A	05-02-94
		MX 25532 A	01-10-93
		WO 9116068 A	31-10-91
		US RE35338 E	24-09-96
		US 5246707 A	21-09-93
US 5364633 A	15-11-94	EP 0672410 A	20-09-95
		JP 7323222 A	12-12-95
		US 5411744 A	02-05-95
WO 9420072 A	15-09-94	CA 2091152 A	06-09-94
		AU 676279 B	06-03-97
		AU 6225394 A	26-09-94
		EP 0687172 A	20-12-95
		FI 954143 A	19-10-95
		JP 8507515 T	13-08-96
		NO 953461 A	06-11-95
		NZ 262541 A	24-04-97